7. TRANSMISSION ELECTRON MICROSCOPIC STUDIES ON PARTIALLY DEGRADED POLLEN GRAINS OF PHOENIX SYLVESTRIS LINN.

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Abstract

Pollen grains of *Phoenix sylvestris* L. were partially degraded with the help of 2-aminoethanol and potassium permanganate and were investigated with the TEM. Six stages of degradation in pollen wall were studied. Linear and cyclic molecules arranged in pentagonal and hexagonal symmetry were observed in the degraded ectexine. On the tectal surface more or less radially oriented linear molecules were observed. The strongest degradation was achieved after treating the pollen with 2-aminoethanol for 72 hours and with aqueous diluted $KMnO_4$ for 24 hours.

Key words: Palynology, *Phoenix sylvestris*, experimental TEM.

Introduction

Extant pollen of *Phoenix sylvestris* L. from India were investigated to observe the molecular structure in partially degraded exine. Significance of these studies lies in observing the resistance of fossil pollen exines at the time of diagenesis. Pollen grains having affinity with those of recent palms are profusely recorded in Tertiary sediments over the world. Experimental studies on recent palm pollen of *Phoenix sylvestris* were carried out to note the changes in exine at molecular level as a result of treatment with 2-aminoethanol. Recent experiments have shown that changes in exine molecules largely depend upon the resistance of pollen walls which varies considerably from species to species (KEDVES and GÁSPÁR, 1994a,b, KEDVES et al., 1998, etc.). Also, the resistance of different layers of exine is variable and differential degradation can be achieved by using chemicals at various stages (FAEGRI, 1956, SOUTHWORTH, 1974, 1985a,b, 1986a,b, ROWLEY, 1978, 1990, 1995, ROWLEY and PRIJANTO, 1977, AUDRAN, 1981, ABADIE, HIDEUX and ROWLEY, 1986-1987).

Present study was undertaken as a collaborative research under the Exchange of Scientist Programme between Hungarian Academy of Sciences, Budapest and Indian National Science Academy, New Delhi.

Materials and Methods

The polleniferous material for investigation was collected from plant growing in natural habitat in eastern Uttar Pradesh, India. For each experiment 5 mg pollen-material was used. Details of the experiments are as follows:

1/7 - 1314 1 ml 2-aminoethanol, duration 24 hours
1/7 - 1315 1 ml 2-aminoethanol, duration 48 hours
1/7 - 1316 1 ml 2-aminoethanol, duration 72 hours
1/7 - 1317 1 ml 2-aminoethanol, duration 24 hours
10 ml 0.01 M $KMnO_4$, aq. dil., duration 24 hours
1/7 - 1318 1 ml 2-aminoethanol, duration 48 hours
10 ml 0.01 M $KMnO_4$, aq. dil., duration 24 hours
1/7 - 1319 1 ml 2-aminoethanol, duration 72 hours
10 ml 0.01 M $KMnO_4$, aq. dil., duration 24 hours

Temperature for all experiments was 30 °C.

For TEM investigations the degraded pollen grains were fixed with OsO_4 aq. dil., dehydrated and embedded in Araldite (Durcupan, Fluka). The ultrathin sections were made on Porter Blum ultramicrotome in the Electron Microscopy Laboratory of the Institute of Biophysics, Biological Research Center of the Hungarian Academy of Sciences, Szeged. The microphotographs were taken on Opton EM - 902 (resolution 2-3 Å) and on Tesla BS - 540 (resolution 6-7 Å).

Results

Experiment No. 1/7 - 1314 (Plate 7.1., plate 7.2., figs. 1,2)

Degradation of protoplasm started. Protoplasmic exudation through the aperture is observed. Electron dense granular areas are clearly discernible in the protoplasmic contents. Characteristic light zone, probably at the place of intine, noticeable between the degraded ectexine and the protoplasm. Degradation of ectexine also started (Plate 7.2., fig. 2).

Experiment No. 1/7 - 1315 (Plate 7.2., figs. 3,4)

Degradation of protoplasm, as observed after the previous experiment, is noticed. Ectexine degradation is a little advanced. Sometimes fine granular structures are in the tectum (Plate 7.2., fig. 4).

Experiment No. 1/7 - 1316 (Plate 7.3., figs. 1-3., plate 7.4., fig. 1)

Protoplasm is completely destroyed and partial degradation of ectexine is noticed. The degraded ectexine at this stage enables the study of different kinds of molecular arrangements in the sporopollenin. Linear and cyclic molecules arranged in pentagonal or hexagonal symmetry are clearly observed (Plate 7.3., fig. 3).

On tectal surface more or less radially oriented linear molecules are observed (Plate 7.3., fig. 2). In Angstrom dimension several kinds of biopolymer units are also observed. Regular pentagonal unit in Angstrom dimension is observed (Plate 7.4., fig. 1).

Experiment No. 1/7 - 1317 (Plate 7.4., figs. 2-4, plate 7.5.)

The molecular structure and the larger biopolymer units are seen. Large number of cyclic structures are noticed at the tectal surface (Plate 7.4., fig. 3). Different kinds of cyclic molecules and cluster of molecules are also seen (Plate 7.5). Biopolymer structures in Angstrom dimension are not observed. Disintegration of molecular system is observed in some parts.

Experiment No. 1/7 - 1318 (not illustrated)

Results of this experiment are identical to those of the previous experiment.

Experiment No. 1/7 - 1319 (Plate 7.6., figs. 1-3)

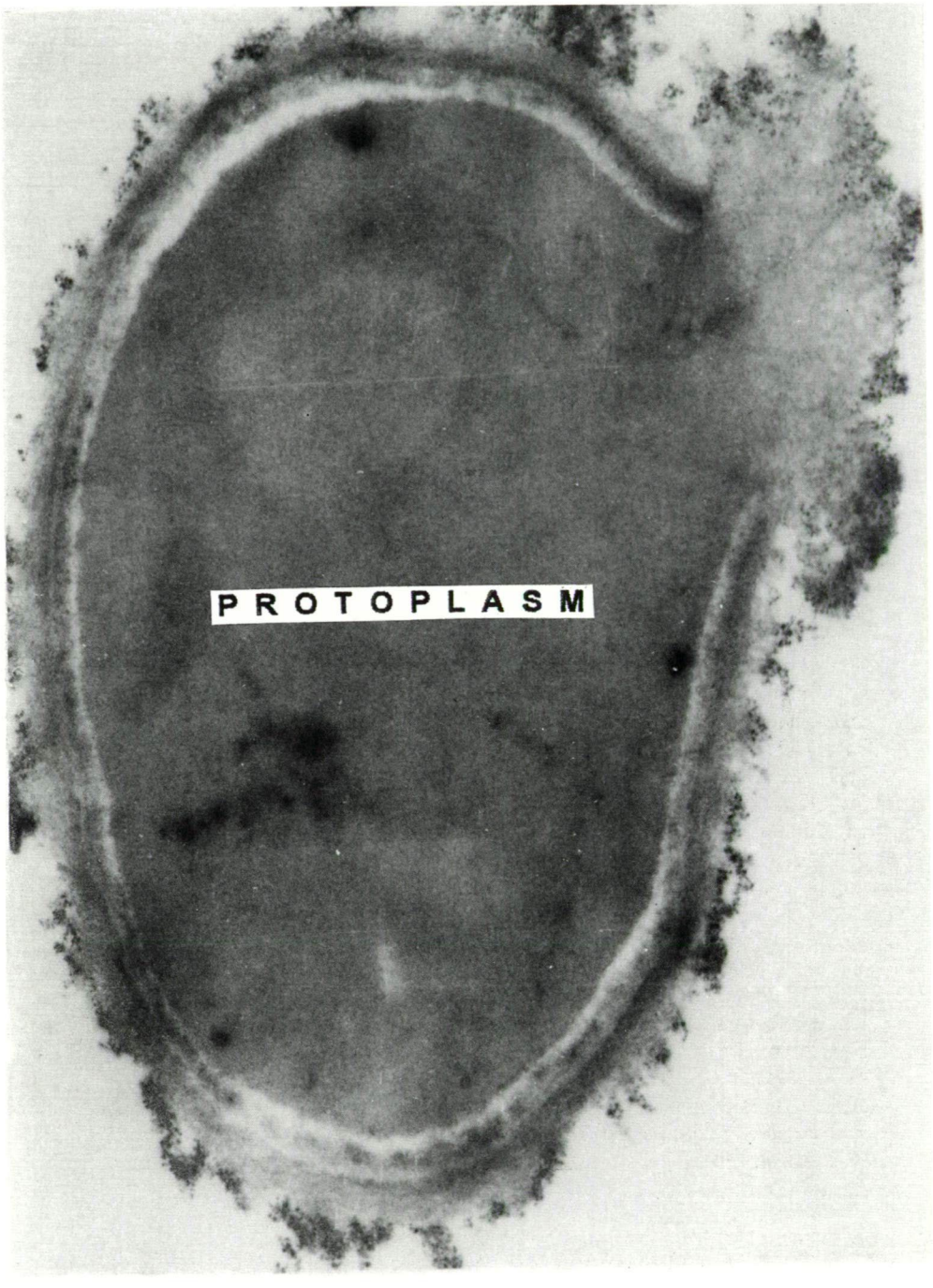
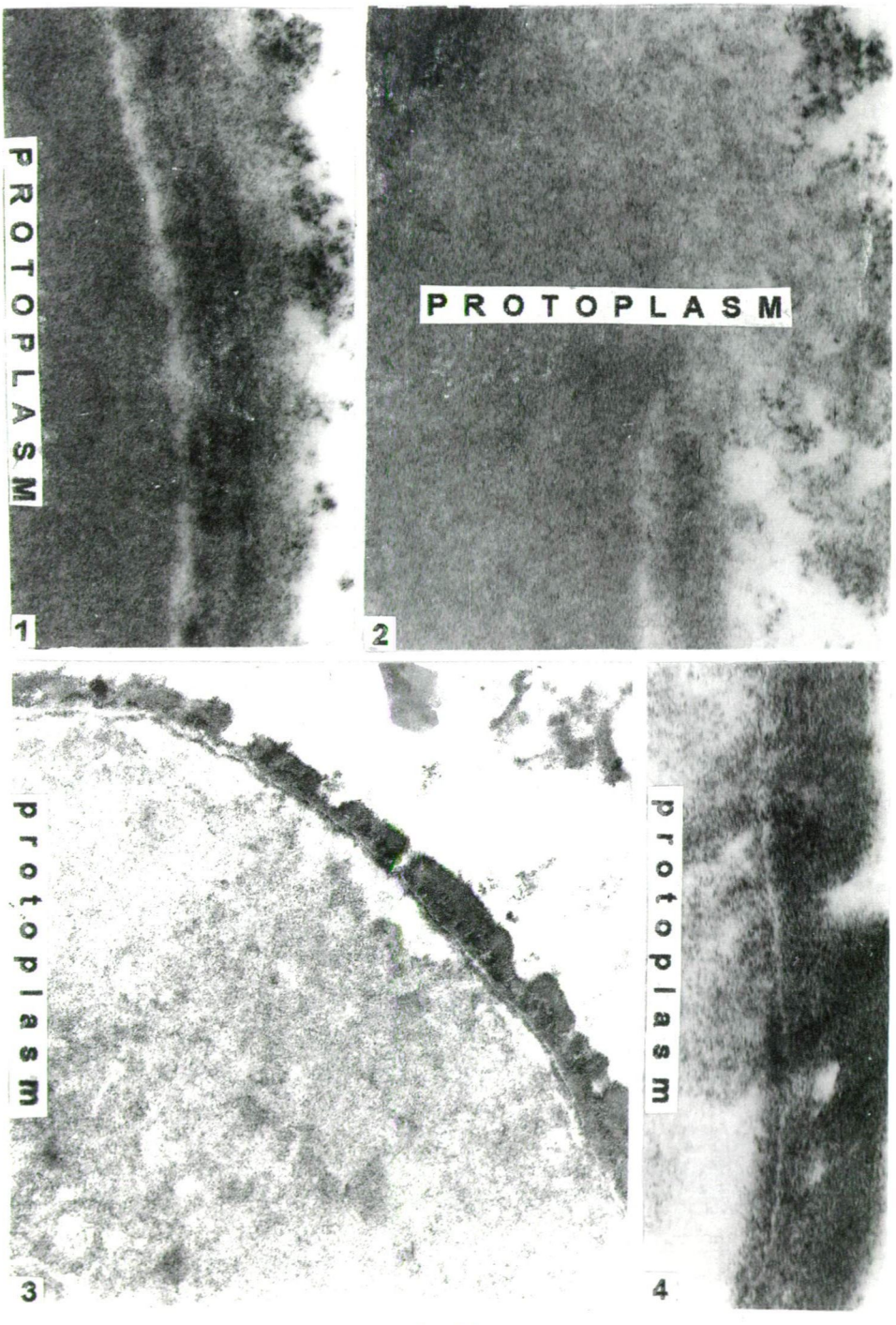


Plate 7.1.



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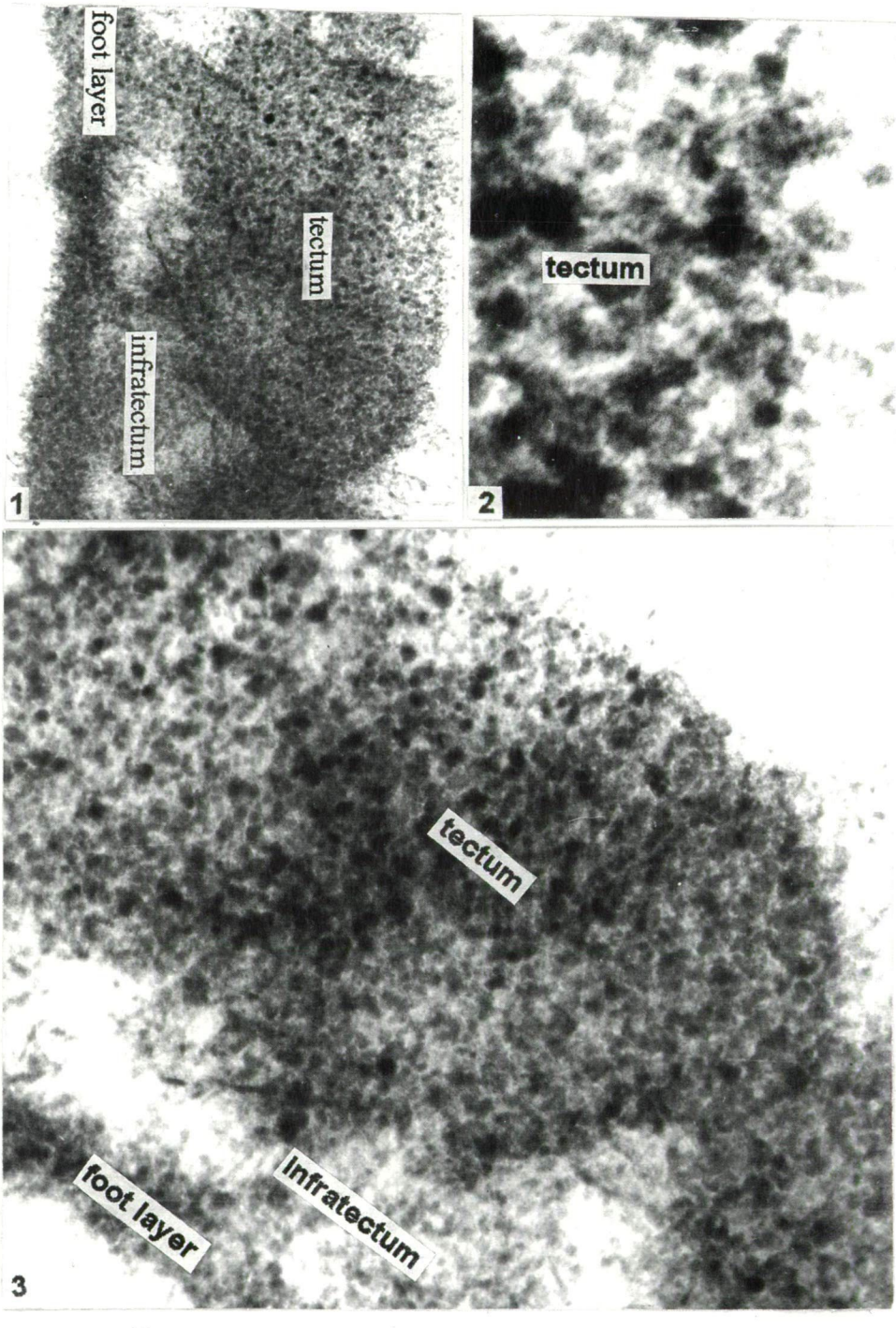
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Plate 7.3.

The strongest degradation is observed. Several linear molecular structures were also noticed (Plate 7.6., fig. 3) which may be part of the stabilizing molecular system of the metastable quasi-crystalloid skeleton. Remnants of the biopolymer skeleton in Angstrom dimension is also seen (Plate 7.6., fig. 2).

Discussion and Conclusions

Degradation of exine with the help of 2-aminoethanol has resulted into observation of substructures below the surface of sporopollenin (SOUTHWORTH, 1974, ROWLEY, 1995, ROWLEY et al., 1981). These substructures are radially oriented and are disposed at right angles to the lamellations of the endexine in *gymnosperms* and *angiosperms* and exospores of *pteridophytic* spores (ROWLEY, 1995). Experimental studies have demonstrated two levels of degradative resistance in different components of the exine as a response to various chemical treatments. The polymerization level of sporopollenin may be one of the reasons for differential resistance between new and old components of exine. Exine of mature pollen when dissolved in 2-aminoethanol results into separation of dializable autofluorescent sporopolleninous component and non-dializable in situ filamentous or fibrillar fraction. With use of preferential staining methods the filamentous fraction, termed as glycocalyx elements, has been demonstrated to be composed of polysaccharides and proteins. The fibrillar elements of the pollen wall get encapsulated by the sporopollenin giving rise to characteristic exinal morphologies. Degradation of exine with 2-aminoethanol at low temperature enables the study of biopolymer structures and their arrangement as it does not completely erode the sporopolleninous component.

Plate 7.1.

Phoenix sylvestris L.
Experiment No. 1/7 - 1314. Ultrastructure microphotograph of the pollen grain. Negative no: 7056, 7.500x.

Plate 7.2.

Phoenix sylvestris L.
1,2. Experiment No. 1/7 - 1314.
1. Details of the partially degraded exine and protoplasm. Negative no: 7059, 50.000x.
2. Highly magnified part of exuding protoplasm. Negative no: 7057, 50.000x.
3,4. Experiment No. 1/7 - 1315.
3. Details of the ultrastructure of the pollen grains. Negative no: 7087, 15.000x.
4. Details of the ultrastructure of the ectexine. Negative no: 7085, 50.000x.

Plate 7.3.

Phoenix sylvestris L.
1-3. Experiment No. 1/7 - 1316.
1. Biopolymer units of the partially degraded ectexine. Negative no: 7096, 150.000x.
2. Superficial molecular structures of the tectum. Negative no: 7078, 1.000.000x.
3. Molecular structure of the ectexine. Highly magnified, showing details of fig. 1. Negative no: 7077, 250.000x.

During the present study biopolymers were observed in the ectexine when pollen grains were treated with 2-aminoethanol coupled with mild heating at 30 °C and without using potassium permanganate. In some experiments (1/7 - 1317 to 1/7 - 1319) dil. aqueous $KMnO_4$ was also used. Studies have demonstrated that the organization of the molecules is mostly cyclic but linear structures are also observed (Plate 7.3., fig. 3). These structures are arranged in pentagonal or hexagonal symmetries. Some elongated structures were also noticed which are possibly helical in nature (Plate 7.3., fig. 2).

Regular pentagonal molecules in Å dimension will enable the study of symmetry in biopolymer organization. Highly organised structures and clusters of molecules were observed (Plate 7.5). Results of the present study indicate that the sporopollenin in ectexine is comparatively resistant. The TEM studies document that ectexine of fossil and recent Palm is compact and homogeneous (THANIKAIMONI, 1970, HARLEY, 1990, FERGUSON and HARLEY, 1993, ZAVADA, 1983). Our study has confirmed these results.

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Plate 7.4.

Phoenix sylvestris L.

1. Experiment No. 1/7 - 1316. Regular pentagonal biopolymer unit. Negative no: 7083, 2.000.000x.

2-4. Experiment No. 1/7 - 1317.

2. Details of partially degraded ectexine. Negative no: 7084, 150.000x.
3. Superficial biopolymer structure. Negative no: 7089, 150.000x.
4. Details of partially degraded ectexine. Highly magnified part of figure 2. Negative no: 7085, 250.000x.

Plate 7.5.

Phoenix sylvestris L.

Experiment No. 1/7 - 1317. Molecular system of different organization levels of the partially degraded ectexine. Negative no: 7088, 2.000.000x.

Plate 7.6.

Phoenix sylvestris L.

1-3. Experiment No. 1/7 - 1319.

1. Ultrastructure and biopolymer system of the partially degraded ectexine. Negative no: 7097, 150.000x.
2. Biopolymer units of the partially degraded ectexine. Negative no: 7099, 1.000.000x.
3. Linear biopolymer units of the partially degraded ectexine. Negative no: 7093, 250.000x.

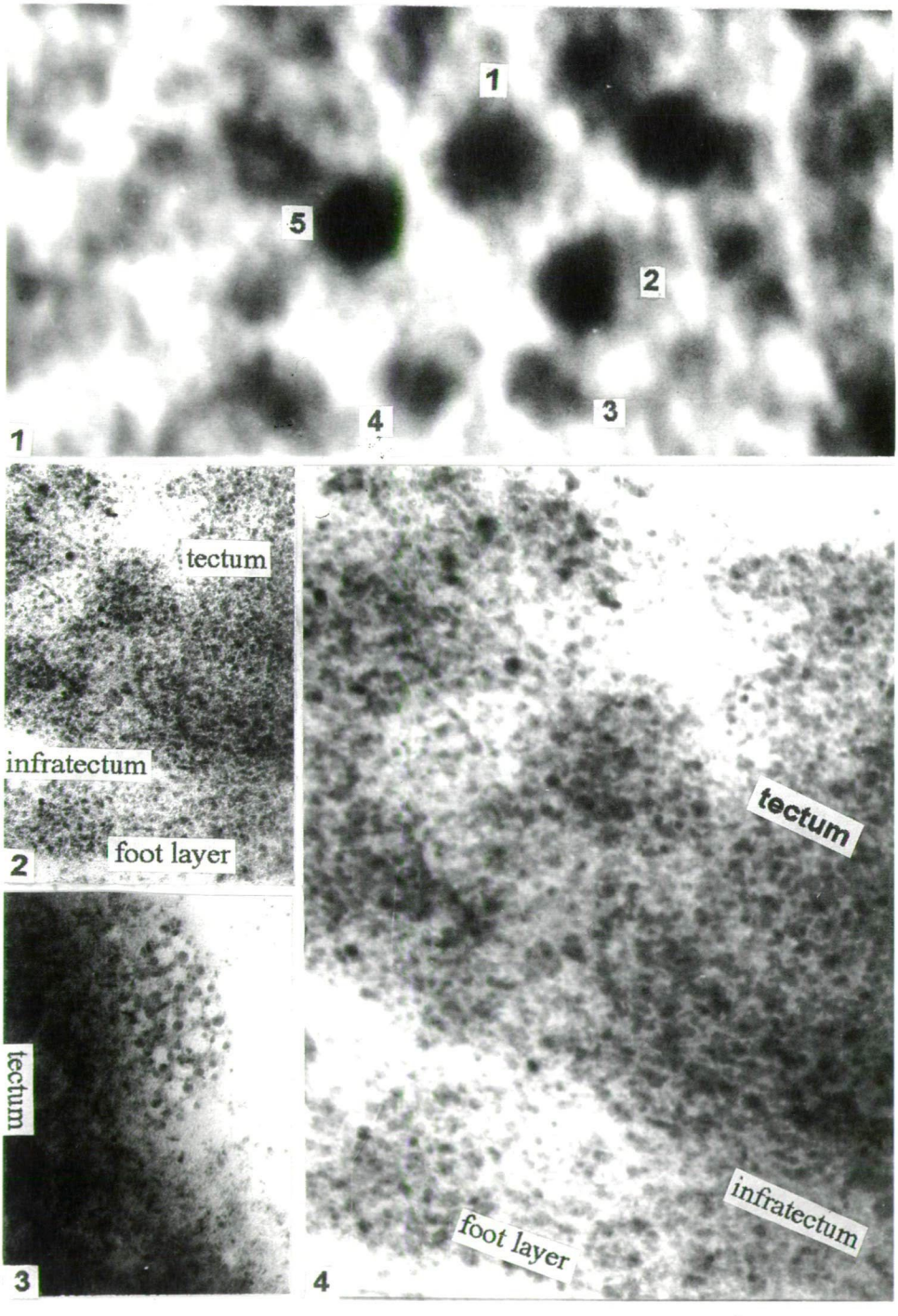
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Plate 7.4.

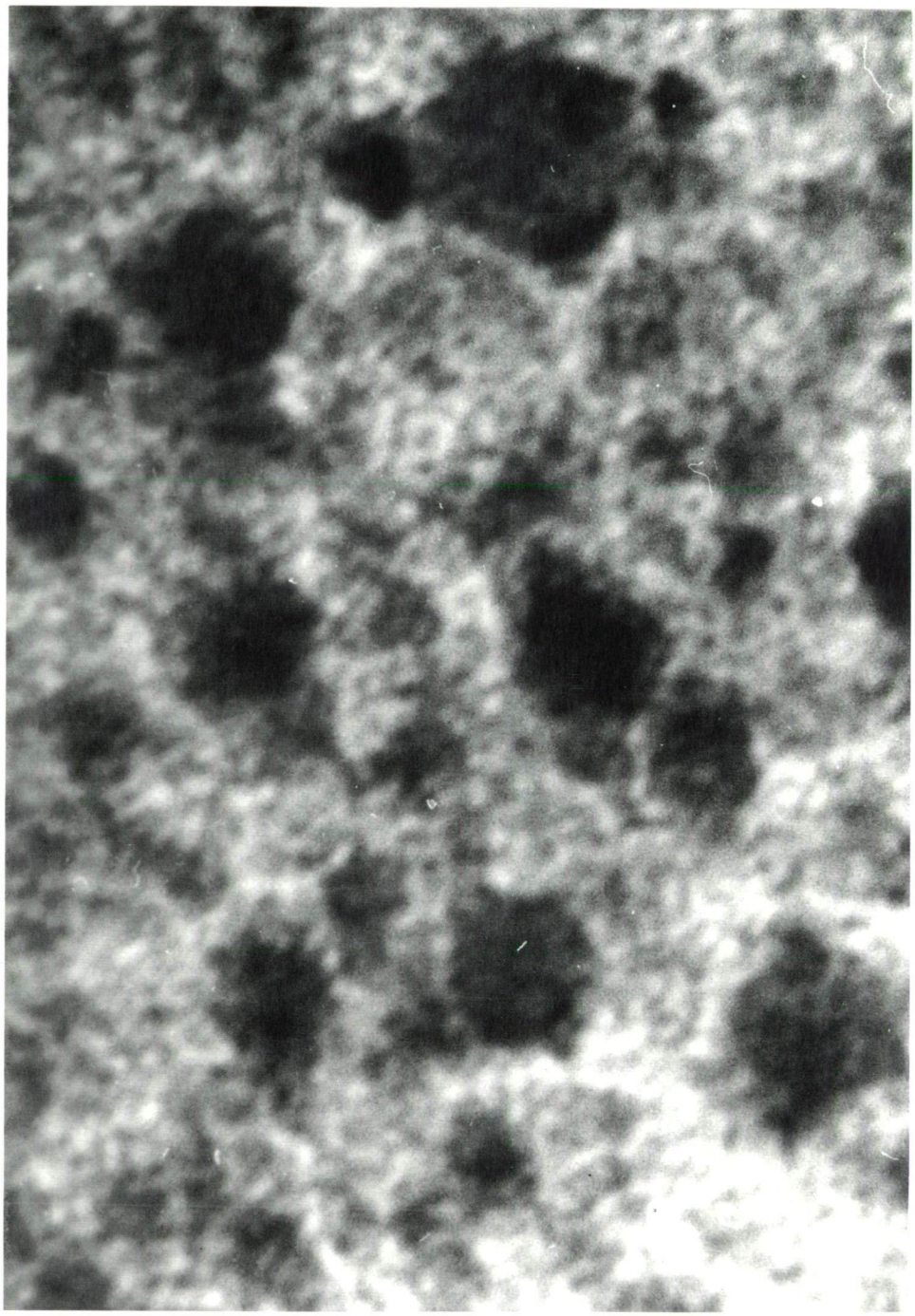

Plate 7.5.

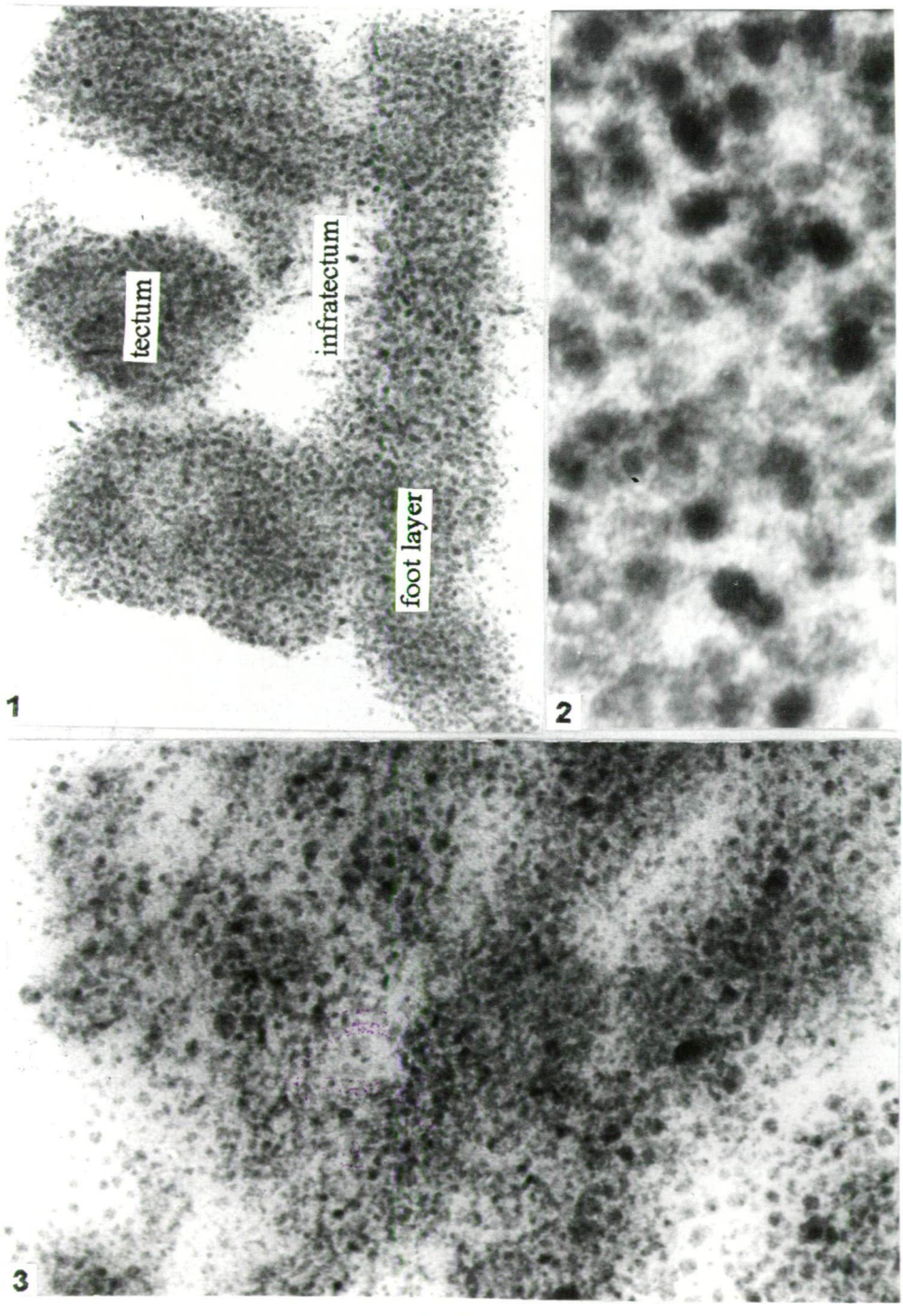

Plate 7.6.

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